Tuesday, July 26, 2005

14:30 Fine mapping of resistance genes in Cocoa

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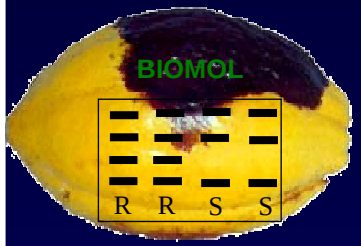
To understand the complexity of the relationship between cocoa and the mains diseases as the witches´ broom disease, one must make reference to an investigation domain that touches at the same time the plant and the fungus. Briefly, generalities about genomic context in cocoa researches are presented and more precisely the characterization of the QTLs identified.

An example is given on the detection and the characterization of QTLs of resistance to *Phytophthora spp*. Different studies on this topic have permitted to show the relation between the genetic map and the physical map, for the regions of the genome involved in the resistance to this disease, implanted in all world cocoa production area.

The strategies in order to characterize the QTLs of resistance to witches´broom disease (*Crinipellis perniciosa*) are also presented. The fine mapping of the major QTL, identified in the F2 (Scavina-6 x ICS1) population, is considered. The goal of this study is to achieve positional cloning of resistance genes coming from the parent Sca6.



Characterization of QTL and fine mapping of resistance genes in cacao

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Breeding and Molecular Biology

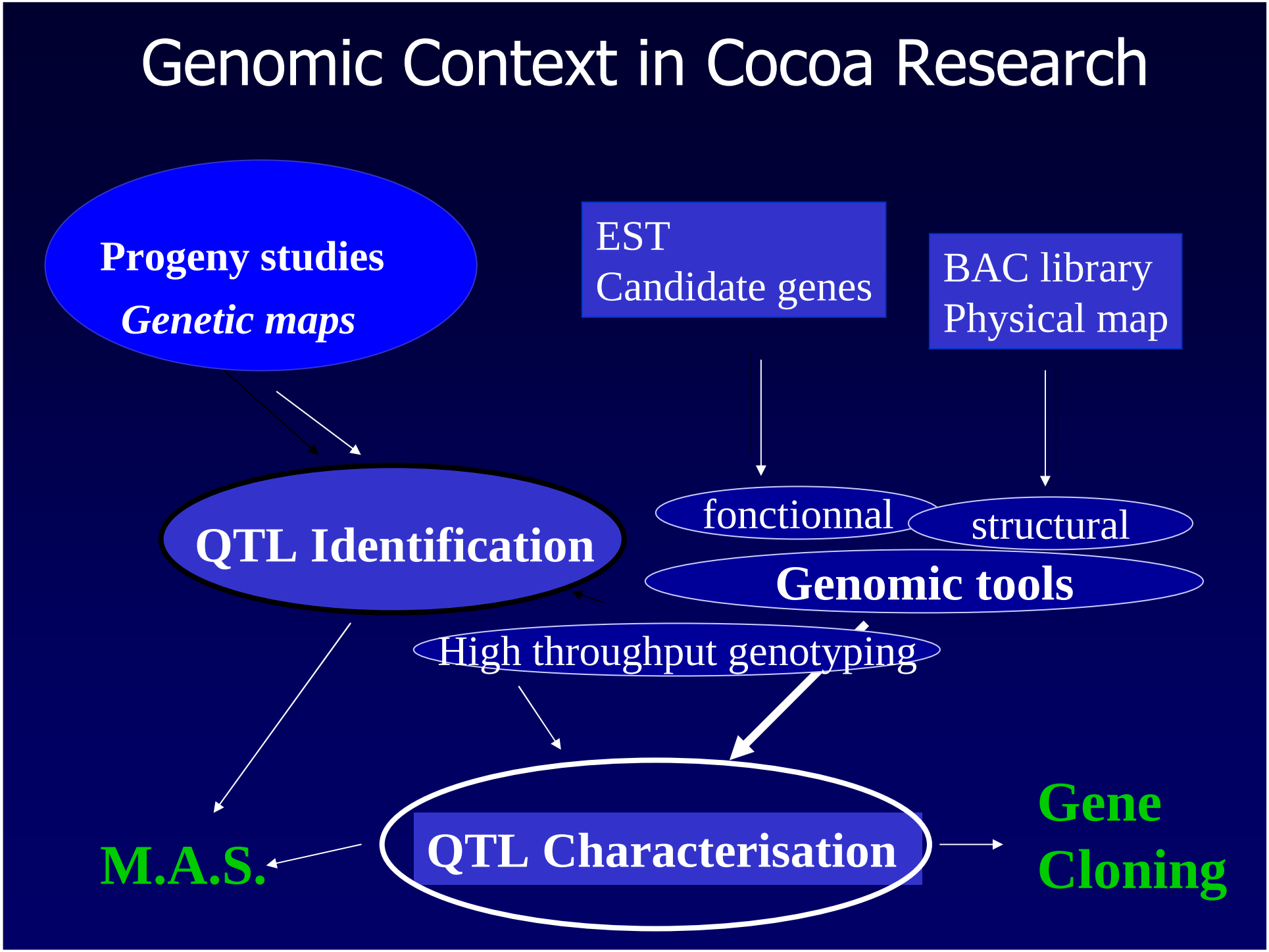
CIRAD/CEPEC Brazil

Challenges for Cocoa Breeding : To obtain a sustainable resistance to Cocoa diseases

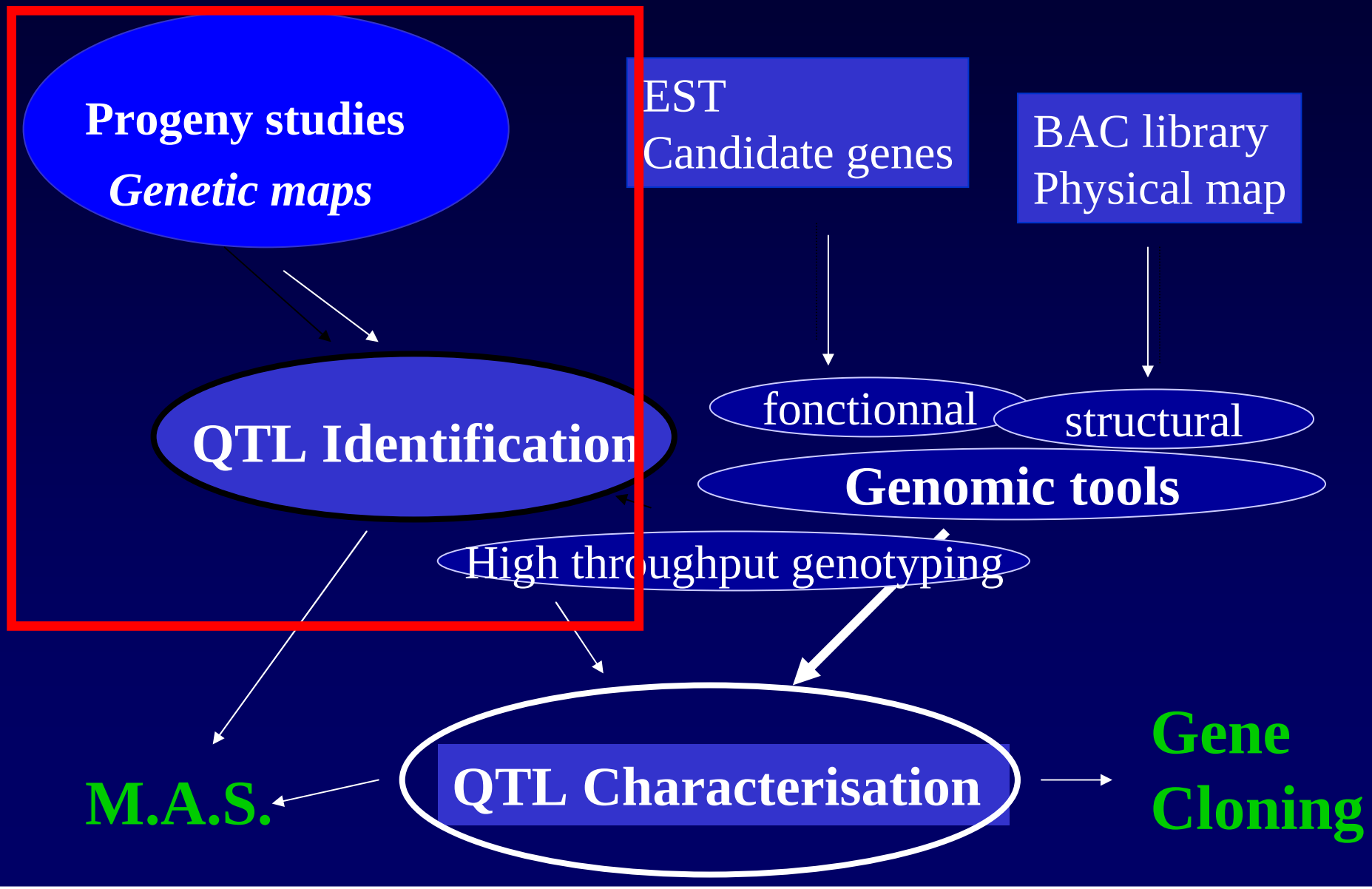
- Need to accumulate favorable alleles in new varieties
- Classical genetic improvement must be strengthened by genomic approach

- Genomic Context in Cocoa research
- Characterization of the QTL involved in Black Pod Disease Resistance
- Strategies for, the Characterization of the QTL and for the Genetic and Physical mapping of resistance genes to *Crinipellis perniciosa*

Genomic Context in Cocoa Research



Genetic Maps and QTLs Studies



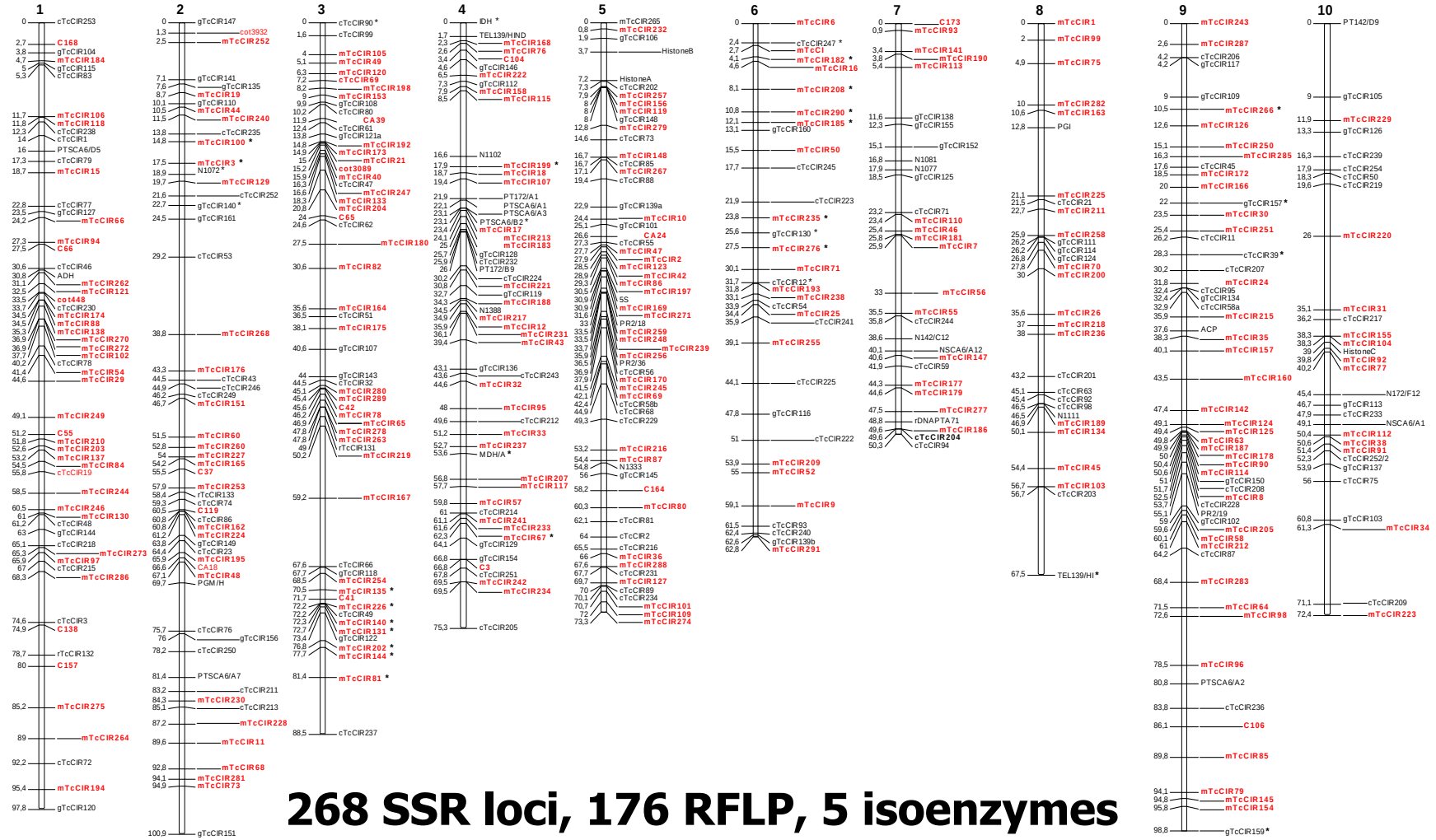
Progenies Studies

- Progenies coming from cocoa breeding program
  - Often with a limited number of trees
  - Possibilities to use experimental design for multi-parent genetic mapping
- Specific progenies has been made for the detection of QTL
  - Number of trees > 200
  - Environmental Approach (Stability and specificity of the QTLs)

Genetic Maps

- Reference genetic map (Pugh et al.2004)
  - 268 SSR
  - 176 RFLP
  - 16 Rgenes
  - 5 isoenzymes
- Numerous genetic maps has been made or are in progress, in different cocoa research centers since 10 years
- Common SRR markers give the possibility, to compare the 10 chromosomes of Cocoa genome of different genetic map populations

Codominating Marker-Based Cocoa Map

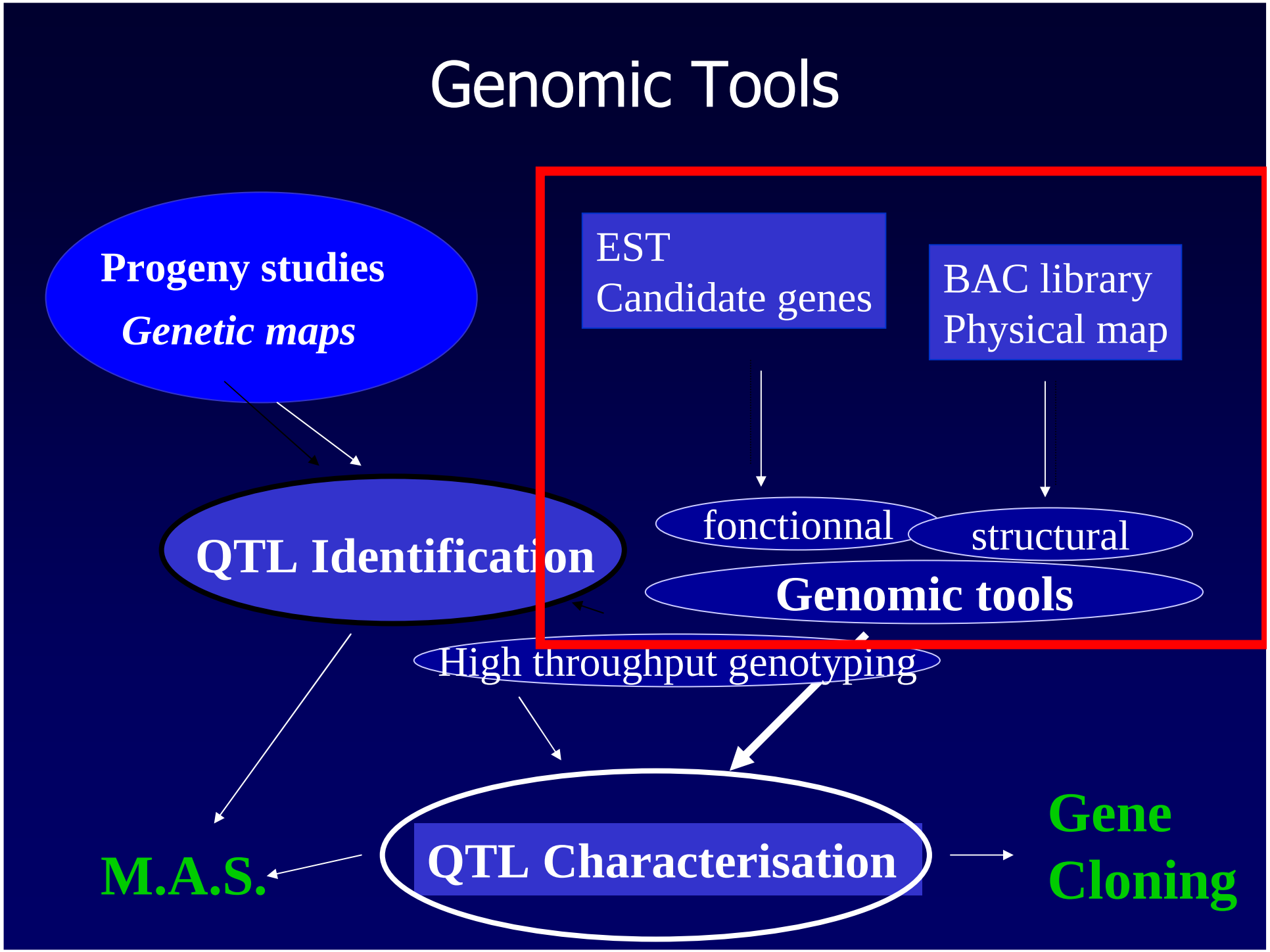


268 SSR loci, 176 RFLP, 5 isoenzymes and 16 Rgenes

QTL of resistance

- Resistant traits
  - Evaluation in the field
  - Evaluation in the nursery or in the laboratory (pod, leaf, meristem, test)
- Resistance be explained by other biological traits not directily involved in resistance (yield components)
- Some cocoa papers about QTL of resistant traits

  Lanaud et al. 1999; Crouzillat et al.2000; Flament et al.2001 ; Queiroz et al.2003; Risterucci et al.2003; Clement et al.2003


Genomic Tools
Progeny studies
Genetic maps
EST
Candidate genes
BAC library
Physical map
QTL Identification
fonctionnal
structural
Genomic tools
High throughput genotyping
M.A.S.
QTL Characterisation
Gene
Cloning

Express Sequence Tag (EST)
Candidate genes approach

- Candidate gene : gene whose the function lets supposed that it is in relation to the expression of the resistance.

- Candidate gene identified on other species
  - Mapping of different Resistance and Defence Genes Analogs (RGA and DGA)
  - (Kuhnd et al.2003; Lanaud et al. 2004)

- Libraries of EST made from the interaction between the pathogen and Cocoa tree
  - Large quantity of EST in the different EST libraries
  - Characterization of EST → Candidate gene

Bacterial Artificial Chromosome (BAC) Library

Physical Mapping approach

Two BAC Libraries in Cocoa :

- From Scavina-6 (resistant to *Phytohpthora* and *crinipellis perniciosa)*. BAC library made by Cirad (Clement et al. 2004)

- From LCT-EET36 (resistant to Moniliophthora roreri). BAC library made by Clemson University for USDA/ARS and Masterfoods.

Construction of the Scavina-6 Bac Library

Vector

Cocoa leaves

DNAextraction

Nuclei DNA isolation

DNA Digestion

DNA digested by enzyme

DNA Ligation

DNA fragments

Transformation into the bacteries

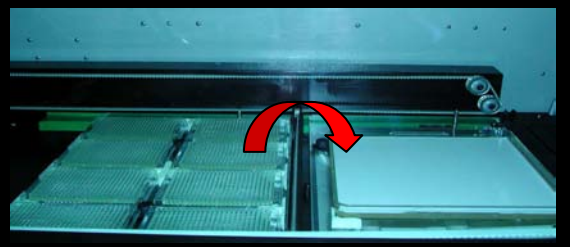
Selection of the bacterial colonies with DNA fragment (BAC)

Colony picking

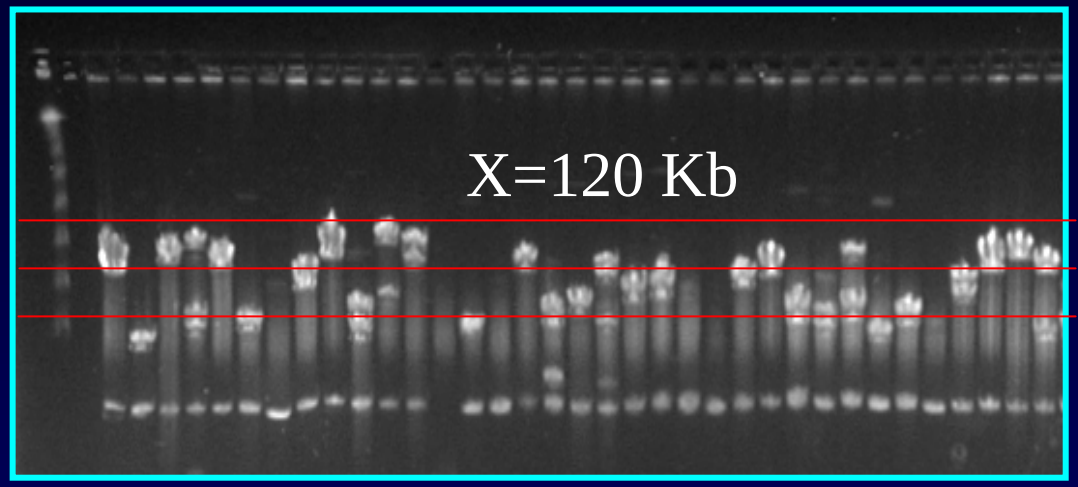
Store individuals BACs in 384-well plates

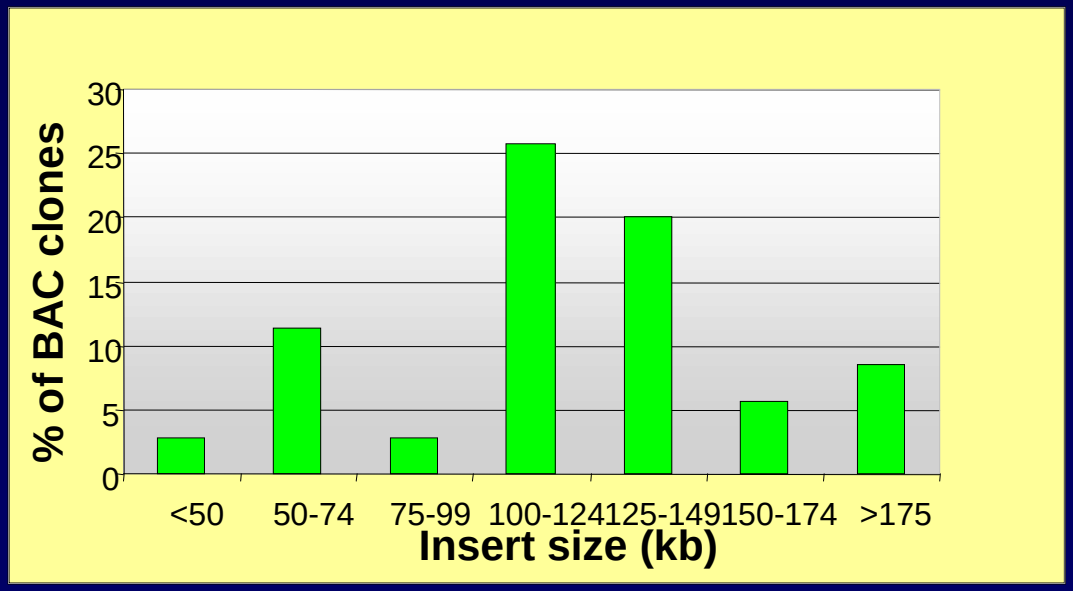
An ordered BAC library

Duplicating on 2 filters



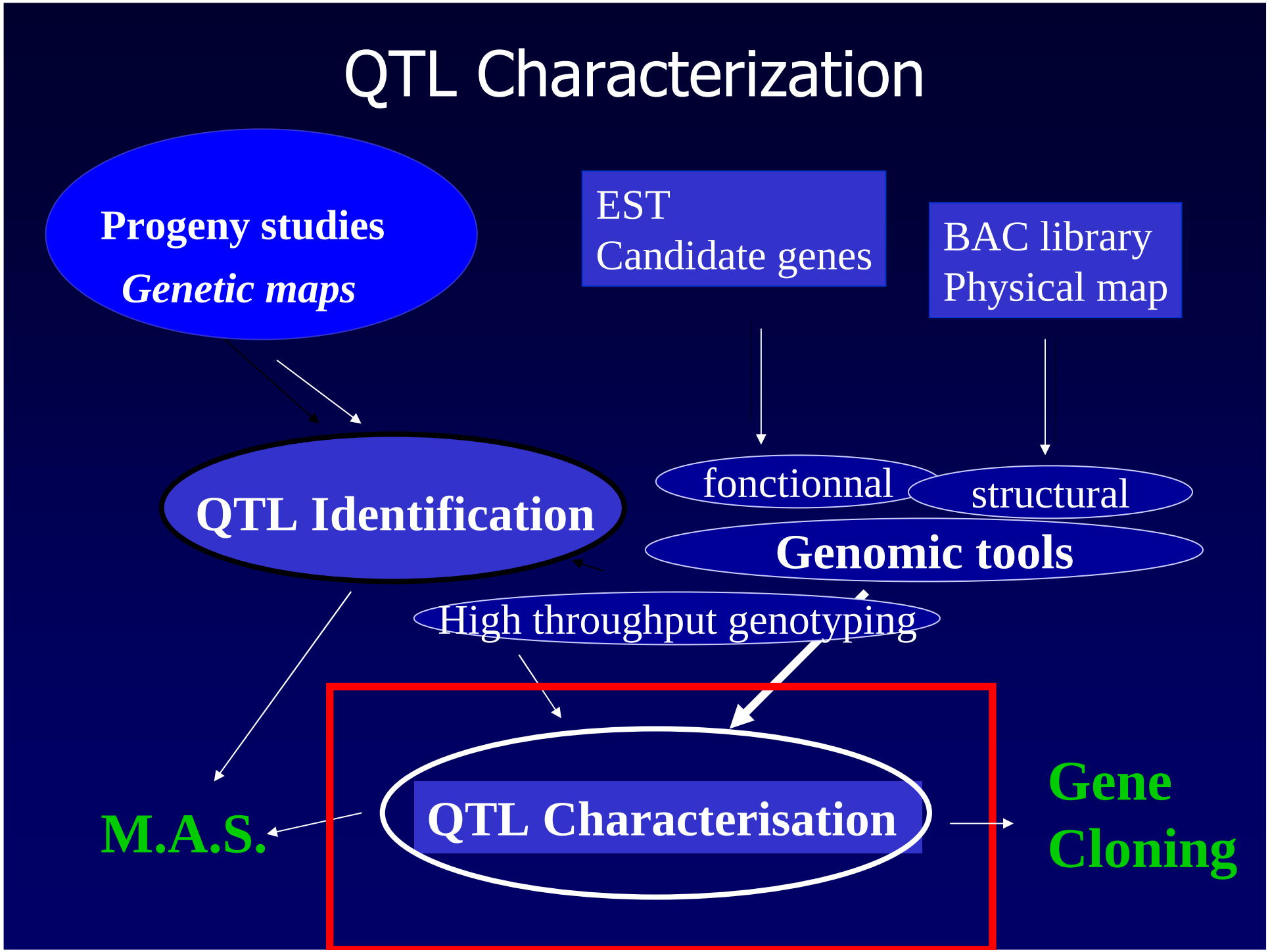
Scavina-6 BAC library Characterization





- HindIII partial digestion
- Number of clones: 36864
- Average size: 120 Kb
- Genome equivalents: 10

(Haploid genome size : $3.9*10^8$ bp)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Characterization of the QTL Involved in Black Pod disease Resistance

Resistance to *Phytophthora*

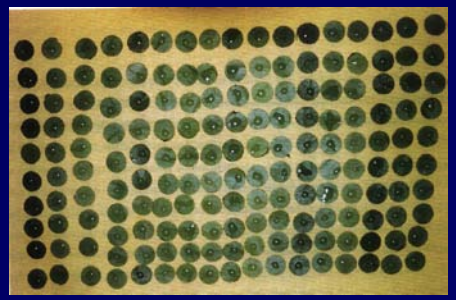
- quantitative resistance
- variable degrees of severity
- several species involved
- several genetic sources of resistance

- Mapping QTL related to resistance traits and to other biologocal traits
- Candidate resistance gene approach
- BAC library screening with cocoa resistance gene analogs

Direct and indirect resistant Traits

- Resistance traits

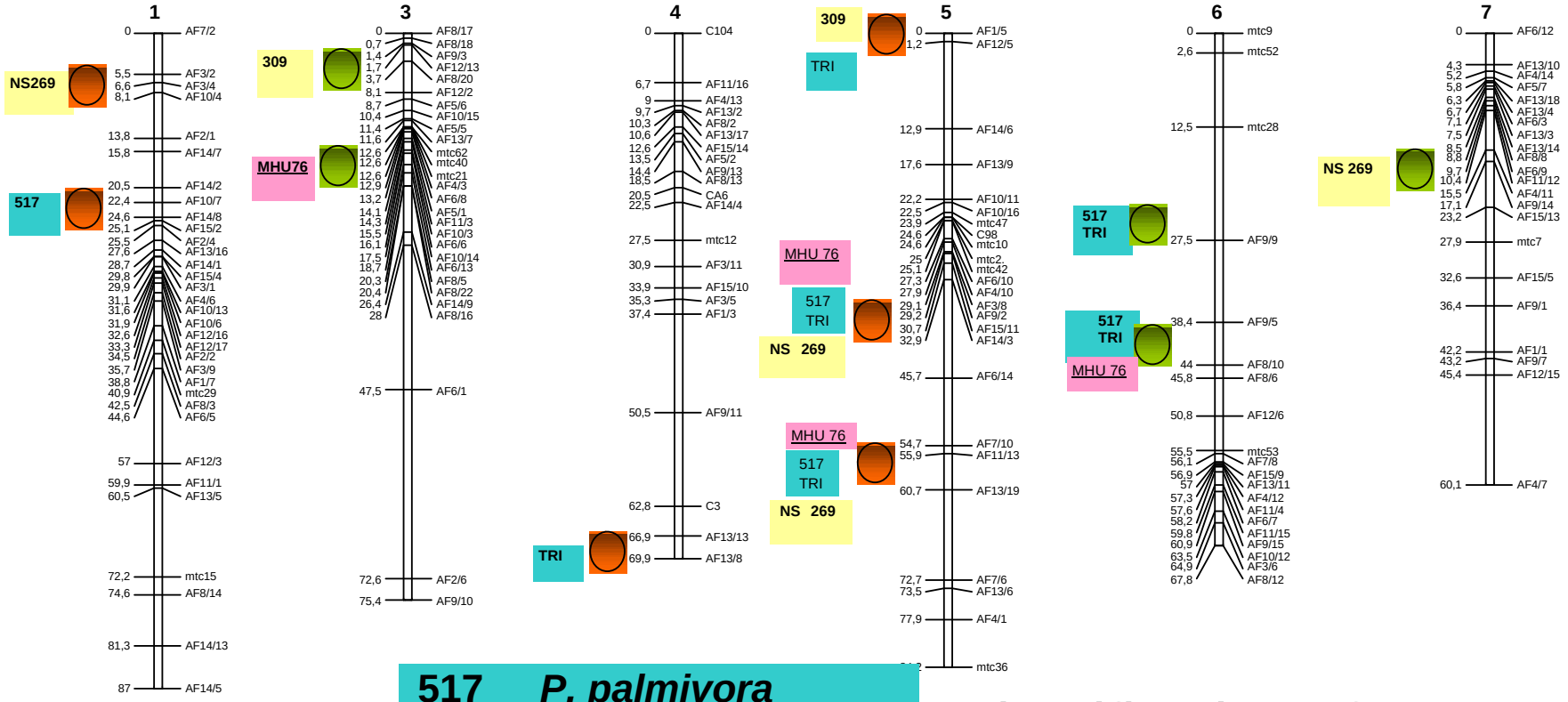
- % rotten pods
- Pod test
- Leaf test (adult leaves)



- Yield factors

- Pod number
- Harvest distribution
- Early yield
- Vigor
  - Foliage densty
  - Trunk circonference

QTLs of Resitance to *Phytopthora* spp



517	***P. palmivora***
TRI	***P. palmivora***
MHU76.2	***P. capsici***
309	***P. megakarya***
NS269	***P. megakarya***

H origin

Sca6 origin

Identification of QTL related to resistance in (**SCA6** x H) x **IFC1**

Candidate Gene Approach

Approach, based on sequence homology existing between resistance genes isolated in different species and cocoa DNA fragments

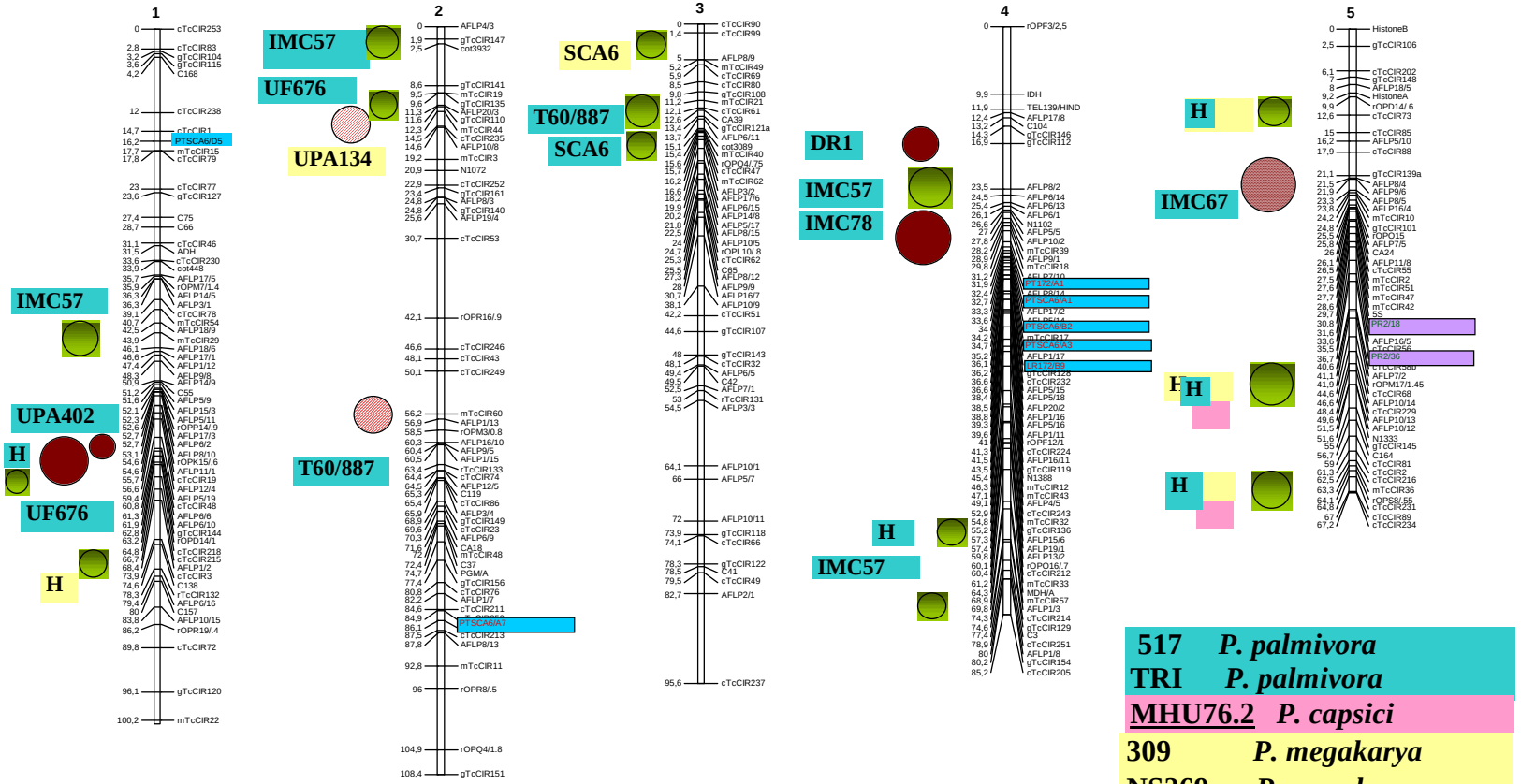
Example : Homology between wheat rust resistance gene And a cocoa DNA fragment

```
Cacao:     16 TTTGGCGCAGTATATAAAGGTAAATTACCCGATGGCCGTCTTGTTGCAGTGAAAGTCCTA 75
              |||||     || || ||||||  |   ||||  ||||     | ||| |||||  ||   | |||
Wheat:   2624 TTTGGAAGTGTGTACAAAGGTGAGCTACCAAATGGAGTGCCTGTGGCAGTCAAGATGCTA 2683

Cacao:     76 AGGGAATCCAAGGGGGACGGAGAGGAATTCATTAATGAAGTGGCCAGCATTAGTAGAACT 135
                | | ||  |   || || |||||     |||||  |||||||| ||  | |||   |    ||
Wheat:   2684 GAGAACTCTACAGGAGAGGGAGAATCGTTCATCAATGAAGTTGCAACCATCGGACTAATC 2743
```

42 cocoa DNA fragments homologous to known resistance or defence genes have been isolated and some of them have been mapped

Candidate Genes Mapping



517	*P. palmivora*
TRI	*P. palmivora*
MHU76.2	*P. capsici*
309	*P. megakarya*
NS269	*P. megakarya*

Candidate resistance genes

Homologous to Pto class genes
Homologous to N class genes
Homologous to PR2 class genes

% ROTTEN PODS

PUTATIVE QTL FOR % ROTTEN

LEAF TEST

FRUIT TEST

Cocoa Homologous Resistance Gene Results

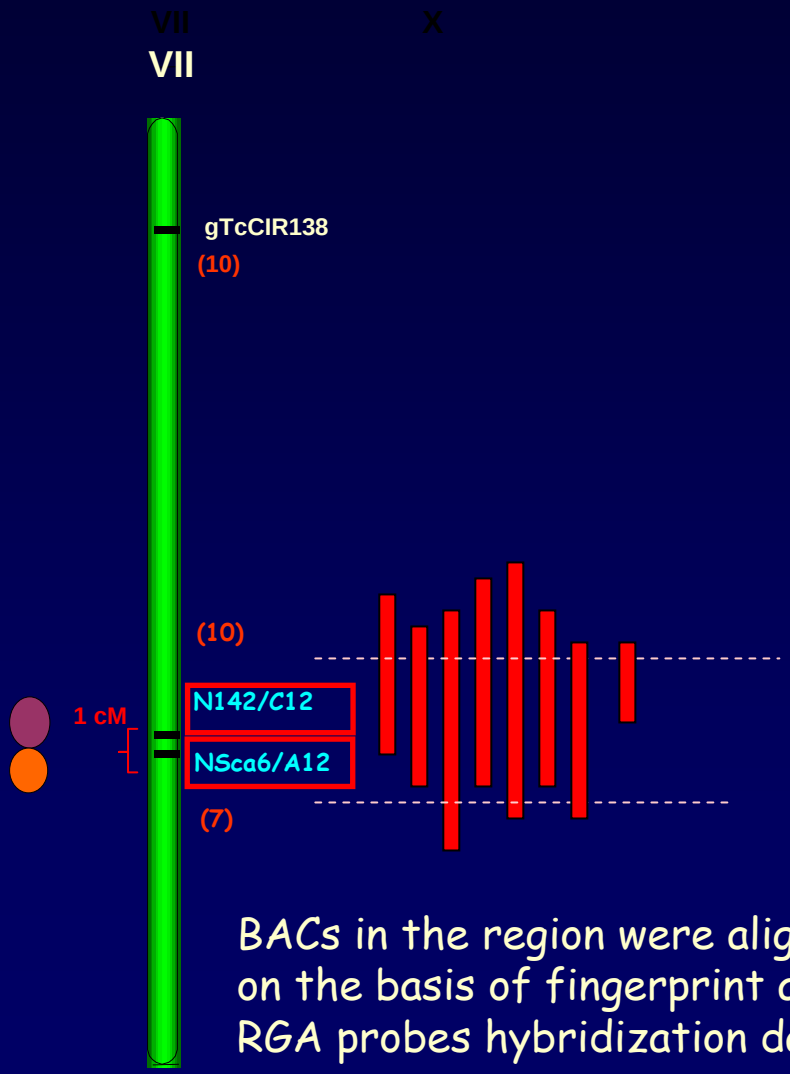
- Colocalisations with QTL of resistance have been observed and some of them could represent candidate resistance genes for cocoa resistance to *Phytophthora spp*

- Larger interest of such candidates genes to study resistance of cocoa to other important cocoa diseases

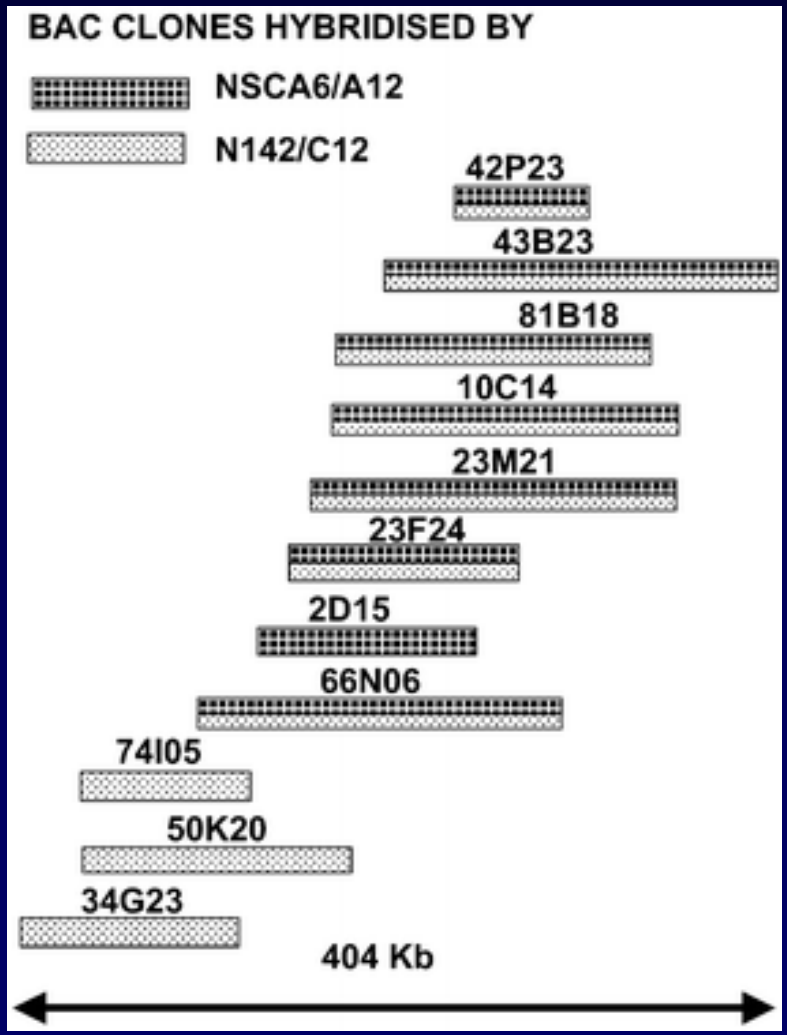
BAC Library Screening with Cocoa Resistance Gene Analogs

- BAC libraries represent a tool to characterize genomic regions containing resistance genes
- Resistance genes are frequently located in cluster in the genome
- The Scavina-6 BAC library was screemed with 9 cocoa Resistant Gene Amalog (RGA)

Contig of BAC clones hybridized by two RGA probes NSCA6/A12 and N142/C12 probes and clustered in a region of 1.1 cM of chromosome VII



BACs in the region were aligned on the basis of fingerprint and RGA probes hybridization data



Total contig 404 Kb

Physical Mapping of RGA Results

- BAC library screening with cocoa RGAs closely clustered in a genomic region previously associated with QTLs for resistance to *Phytotpthora* spp
- First step towards dissecting out the structure of the resistance genes clusters in cocoa

Strategies for, the Characterization of the QTL and for the Genetic and Physical Mapping of Resistance Genes to *Crinipellis perniciosa*

- Candidate gene approach
- QTL resistance (Scavina-6) for witches´broom : fine mapping and physical mapping of this region of the cocoa genome

Candidate Gene Approach
(similar approach to the one previously presented)

- Homolog of resistance genes identified in other species and candidate genes already physically mapped in cocoa genome

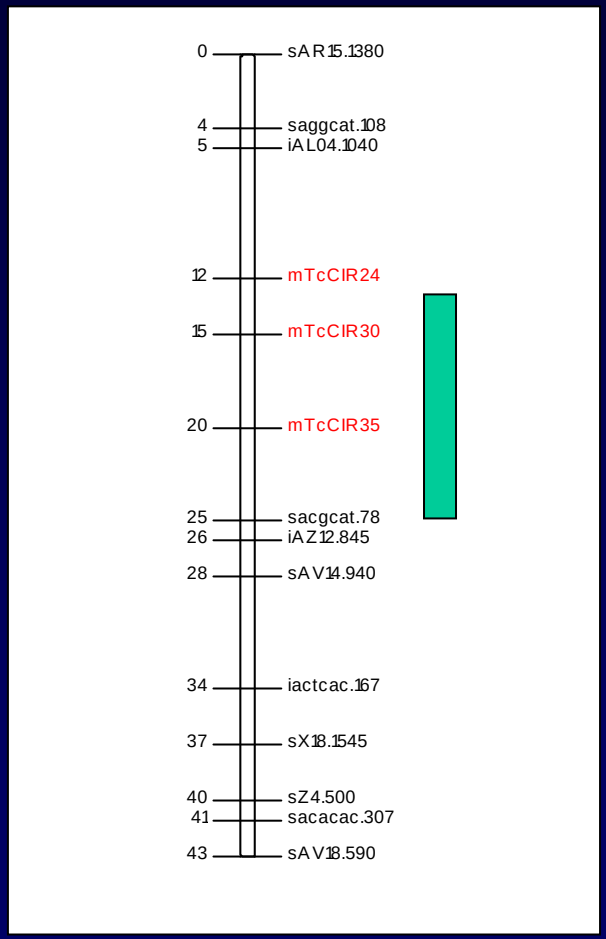
- Candidate genes coming from EST libraries* and made in different context of the interaction : Cacao-*Crinipellis p.*
  - Sequencing of 5000 EST coming from : TSH1188(R)-Crinipellis and Catongo(S)-Crinipellis UESC-CEPLAC
  - Others EST libraries in progress

Fine Mapping of the Main QTL of Resistance to *Crinipellis perniciosa*

- One QTL for resistance to witches´broom has been found with a major effect (F2 scavina-6 xICS1)
- Fine mapping of this region, need of the very large population in segregation
- Objectives
  - To find markers very closed to the QTL (SAM)
  - Cloning the genes

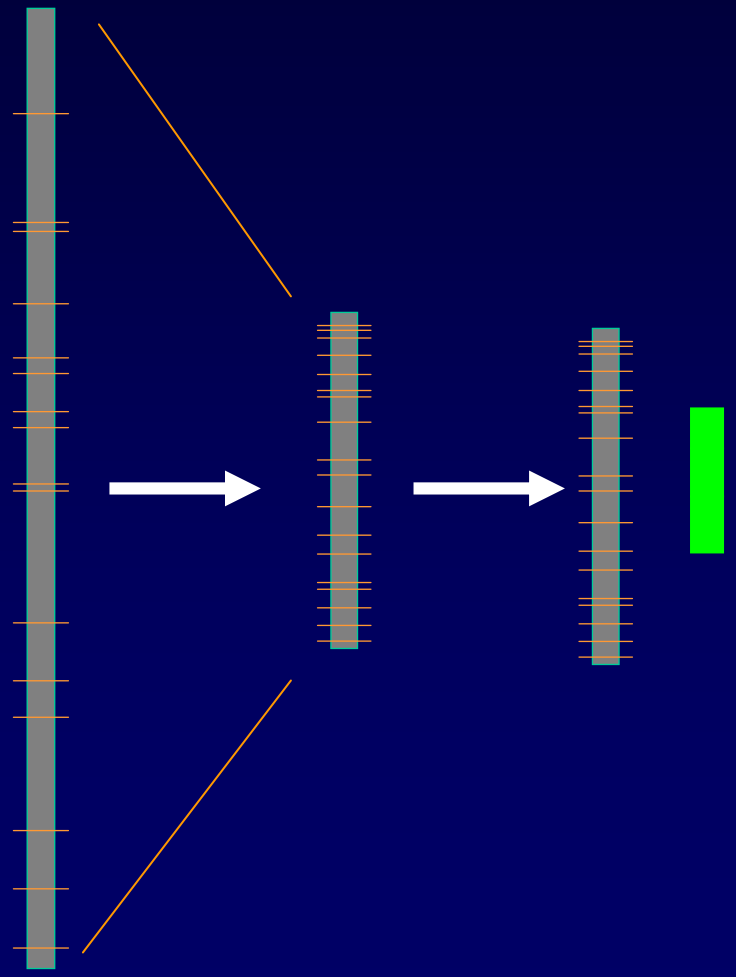
Recombinant Screening of the F2 Scavina-6 x ICS1 Population

- To obtain a population with minimum of 1000 F2 plants (in progress)
- Screening the recombinants with markers strongly linked to the QTL
- To duplicate the recombinant population (mapping and security)



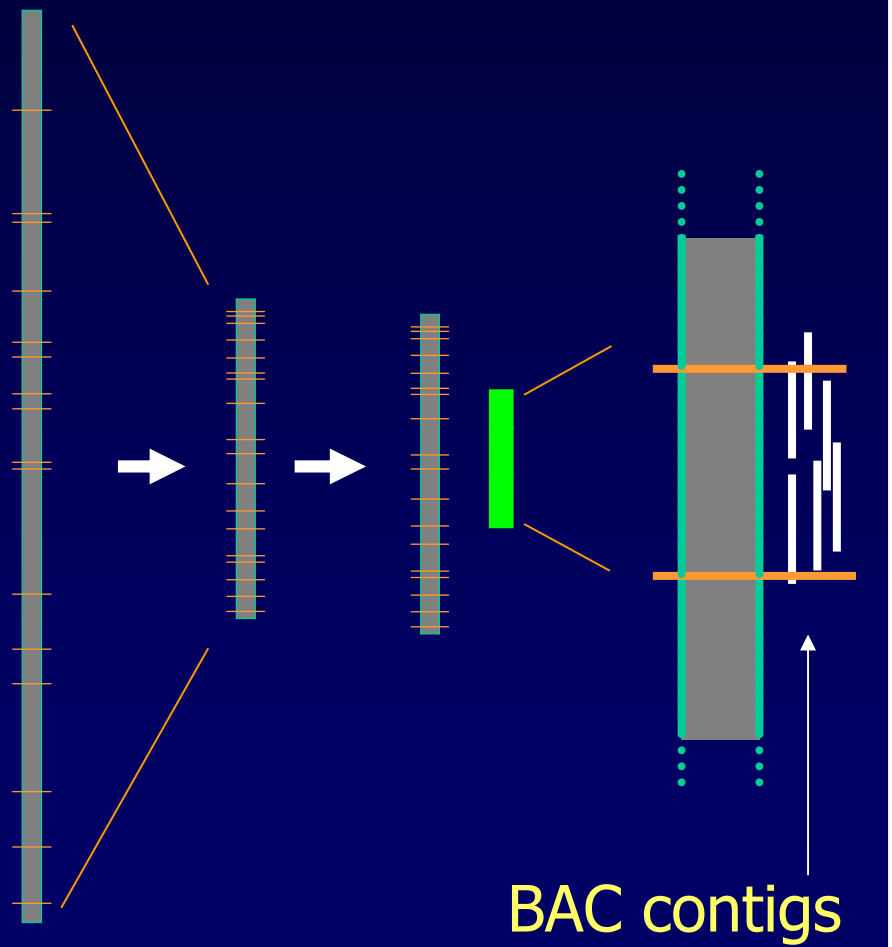
Fine Mapping of the QTL Only with the Recombinant Seedlings

- Fine genetic mapping with new markers (AFLP, SNP)
- Assessment of the level of resistance of the recombinant seedlings
  - Crinipellis inoculation meristem
  - Evaluation of resistance symptoms
- QTL mapping



Physical mapping : Screening of the Scavina-6 BAC clones

- Hybridization of the closely linked markers to the QTL on the Scavina-6 BAC library
- Physical mapping of the QTL region (to ordain the BAC contig)



Following stages

- Hybridization of EST coming from inter action Cacao-Crinipellis libraries with the identifed BAC clones (QTL)
- Isolation and cloning of the potencial resistant gene
- Control of the function of the isolated resistante gene (ARN interference method)